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PHYTOCHEMICAL ANALYSIS AND ANTICANCEROUS ACTIVITY OF SELECTED MEDICINAL PLANTS

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Abstract

Detection of phytoconstituents in plants makes the primary step in drug evaluation. In the present study, preliminary phytochemical studies were conducted on the Andrographis paniculata, Nees., Centella asiatica, L. and Murraya koenigii, L.. Extracts were prepared in ethanol, methanol and distilled water using powdered leaves. For the detection of primary and secondary metabolites preliminary qualitative phytochemical tests were conducted based on standard procedures described by Harbourn. Among the three constituents selected for study, carbohydrate was the major primary metabolite. Alkaloids, tannins and phenols were also present in all. As the present study is qualitative analysis, it helped in detection of various phytoconstituents in the selected plants under study. Anticancerous activity of Andrographis paniculata, Nees., Centella asiatica, L. and Murraya koenigii,L. were studied.

Keywords: ***Phytochemicals, Ethanopharmacology, Andrographis paniculata, Nees., Centella asiatica, L. and Murraya koenigii, L.***

I. INTRODUCTION

The human race since time immemorial has been using plants as a major source of medicine. Medicinal plants play a key role in the health care system of almost all countries. About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials (WHO, 1993).

Traditional medicine refers to a broad range of ancient and natural health care practices including tribal or folk medical practices as well as classical systems of medicine such as Ayurveda, Sidda, Unani and Amachi. These medical practices originated much before the application of modern scientific method. Further, the herbal medicines are also used in self medication in all cultures. Although herbal drugs are being used to treat various ailments very often these drugs are unscientifically exploited and improperly used. Therefore, drugs deserve detailed scientific studies in view of their importance in human welfare.

There are 119 drugs of known structure that are extracted from higher plants and used in allopathic medicine (Farnsworth, 1990). The traditional herbal drugs are to be subjected to scientific verification for their rational and proper use. Ethnopharmacology as a well defined science, developed over the past 16 years, can be defined as the interdisciplinary scientific exploration of biologically active agents traditionally used or observed by man (Bruhn and Holmstedt, 1981). The major objectives of ethnopharmacology are to provide a rational explanation how a traditional medicine works to develop appropriate scientific method of evaluation and standardization and administration.

Further, ethnopharmacological studies can solve the problem of ayurvedic and other traditional drugs having controversial botanic identity.

The Indian subcontinent is endowed with rich and diverse local health traditions which are matched with equally rich and diverse plant genetic resources. The resource base of local health trations is mainly the plants. It is estimated about 7,500 plants are used in local health traditions in mostly rural and tribal villages of India. Out of these the real medicinal value of over 4000 plants are either little known or hitherto unknown to the mainstream population (Pushpangathan, 1995). The classical systems of medicine such as Ayurveda, Siddha, Unani, Amachi and Tibetan use about 1,200 plants (Pushpangathan, 1995).

Many plants are used to treat cancer in ethnomedical practices in different parts of the world. It has been estimated that more than 3000 species of plants have been used throughout the world to treat cancer (Hartwell, 1971). In addition to the flowering plants, mushrooms (Mascarenhas, 1994) and ferns (Rastogi and Dhawan, 1982) have been advacated for the treatment of cancer.

There are some important plant derived anticancer drugs which have passed clinical trials with reasonable efficiency and some levels of safety. They are vinblastin, vincristine (*Cathranthus roseus*), taxoids (*Taxus brevifolia and Taxus baccata*) and phodophyllum and its derivative etoposide (*Phodophyllum* sp.). However, these drugs are also not satisfactory, considering efficiency and side effects.

Biomolecules can be classified into primary metabolites and secondary metabolites. Primary metabolites are primary product of metabolism which are present in all living systems and play an important role in the energy requirement process. Proteins, carbohydrates, lipids and nucleic acids are examples of primary metabolites. Secondary metabolites are produced from primary metabolites. They present only in very little amounts and play a non essential role but are very specialized chemicals doing a specific job of either curing a particular disease, germicidal effect etc. Secondary metabolites are pharmacologically important and they include antibiotics, alkaloids, glycosides, terpenes, flavonoids, saponins, phenols, tannin etc. About one lakh twenty thousand secondary metabolites have been identified. They are stored in different organs of plant and percentage of accumulation also varies from organ to organ. Secondary metabolites make a plant medicinally important.

II. MATERIALS AND METHODS

Three medicinal plants namely, *Andrographis paniculata*, Nees, *Centella asiatica*, L and *Murraya koenigii*, L were selected for phytochemical screening and anticancerous activity detection. The whole plant was used in the case of *A. paniculata* and *C. asiatica*, while the leaves were used in the case of *M. koenigii*. The plant material were washed with water and separate from the root, then dried in shade and powdered by a mechanical grinder and stored in room temperature (Plate 1). Microscopic examinations were conducted for all plants both stem and leaves and histochemical evaluation was done for the detection of starch, lipid and lignin.

The powdered plant materials were extracted with 3 different solutions – Methanol, Ethanol, distilled water. The extract of the samples were prepared by 2g of the dried powder in 10 ml of different solvents. The extracts were filtered using filter paper.

The extracts were tested for the detection of various compounds such as Carbohydrates, Alkaloids, Glycosides, Flavinoids, Tannin, Saponin, Coumarin, Protein and amino acids present in the

plants (Sadasivam *et al*., 1992). Preliminary phytochemical screening indicates the presence of certain compounds. To quantify these compounds various tests are conducted such as carbohydrate (Anthrone method), Proteins (Lowry's method) and Phenol (Folin-ciocalteau reagent test) (Plates 2, 3, 4).

The test compounds were studied for short term in vitro cytotoxicity using Daltons ascites cells (DLA). The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by trypan blue exclusion method. Viable cell suspension was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixture were incubated for 3hours at 37^{O}C. Further cell suspension was mixed with 0.1ml of 1% trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take op dye. The numbers of stained and unstained cells were counted separately (Plate 5).

$$\% \text{ of Cytotoxicity} = \frac{\text{Number of Dead Cells}}{\text{Number of Live Cells+Number of Dead Cells}} \text{ X } 100$$

III. RESULTS

The organoleptic and morphological characteristics of *Andrographis paniculata*, Nees., *Centella asiatica*, L., and *Murraya koenigii*, L. are presented in table 1 and table 2 respectively.

Table 1 - Organoleptic Analysis

Sl. No.	Character of the dried plant material	*Andrographis paniculata,* Nees.	*Centella asiatica,* Linn.	*Murraya koenigii,* Linn.
1	Colour	Dark Green	Greenish yellow	Greenish brown
2	Odour	Aromatic	Aromatic	Aromatic
3	Taste	Bitter	Bitter	Bitter
4	Fineness	Rough	Rough	Rough
5	Surface appearance	Rough	Smooth	Rough

Table 2 - Morphological Comparison

Characters	*Andrographis paniculata,* Nees.	*Centella asiatica,* L.	*Murraya koenigii,* L.
Habit	Herb	stoloniferous perennial herb	tree
Stem	Quandrangular, green	Weak stemmed	Barked stem, dark brown

Leaf	simple, lanceolate, acute at both ends, glabrous, main nerves 4-6 pairs	orbicular, crenatr, palmately nerved deeply cordate with an angular sinus	evergreen, opposite, always gland-dotted
Inflorescence	terminal racemes	umbel	terminal corymbose cymes
Calyx	5, partite, imbricate gamosepalous	Calyx 5	Calyx 5 lobed,
Corolla	Bilabiate, small, pale but blotched and spotted with brown and purple	Petals 5 minute, ovate, minute	5, polypetalous; imbricate
Androecium	Stamens 2, 1 whorled, fertile stamens	Stamens 5, Androecial members free of the perianth,1 whorled.	Stamens 10
Gynoecium	Bicarpellary, syncarpous; superior, bilocular	Bicarpellary, syncarpous; synovarious; inferior.	Syncarpous ovary
Fruit	capsule	a schizocarp	berries subglobose or ellipsoid
Seed	non-endospermic	endospermic	endospermic

3.1 PHYTOCHEMICAL ANALYSIS

In the phytochemical analysis of *Andrographis paniculata*, Nees., *Centella asiatica*, L. and *Murraya koenigii*, L., we observed that the presence of alkaloid, carbohydrate, protein, phenol, etc. It is observed in different extracts such as aqueous, methanol, ethanol. Alkaloid is the main content in *Andrographis paniculata*, Nees. (Table – 3).

Table 3 - Qualitiative Phytochemical Tests

Phytochemical tests	**Aqueous extract**			**Ethanol extract**			**Methanol extract**		
	A.paniculata	*C.asiatica*	*M.koenigii*	*A.paniculata*	*C.asiatica*	*M.koenigii*	*A.paniculata*	*C.asiatica*	*M.koenigii*
1. **Test for alkaloids** a. Wagner's test	+	+	+	-	-	-	-	+	-
b. Mayers test	+	+	+	+	+	+	+	+	+
2.**Test for proteins**	+	+	+	+	+	+	+	+	+

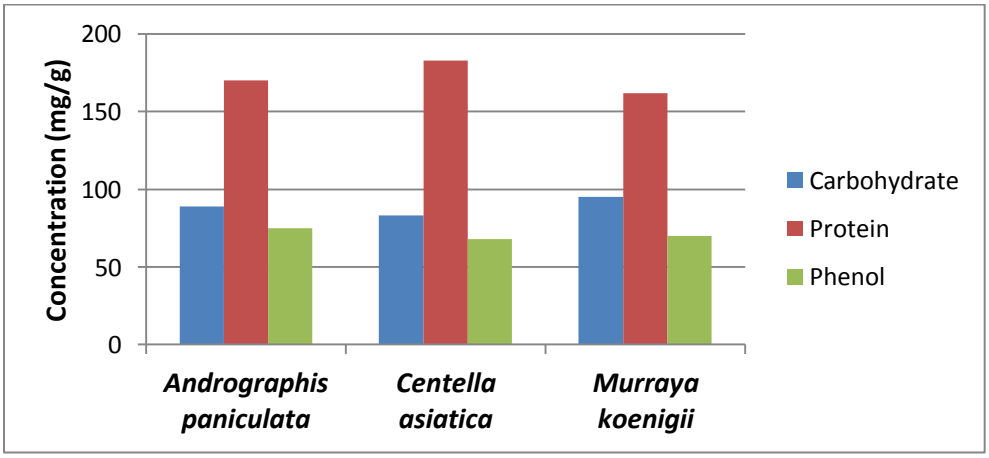
a. Xanthoprotein test b. Ninhydrin test	-	-	-	+	-	-	-	-	-
3.**Test for carbohydrates** a. Fehling's test	+	+	+	+	+	+	+	+	+
b. Benedict's test	+	+	+	+	+	+	+	+	+
4. **Test for flavonoids** a. Ammonia test	-	-	-	-	-	-	-	-	-
b. Pew test	+	+	+	-	+	-	+	+	-
5. **Test for phenol and tannins** a. Ferric chloride test	+	+	-	+	+	+	+	+	+
6. **Test for saponins** a. Foam test	-	-	-	-	+	-	-	+	-
b. Sodium bicarbonate test	-	+	-	-	-	+	-	-	-
7. **Test for glycosides** a. Keller killani test	+	+	+	+	+	+	+	+	+
b. Borntragers test	+	+	+	+	+	+	-	-	-

+ Present, - Absent

3.2 QUANTITATIVE ANALYSIS

The preliminary phytochemical analysis shows the presence of carbohydrate, protein, phenol etc. So it is estimated using different methods. Total concentration of carbohydrates, protein and phenol present in *Andrographis paniculata*, Nees. is 89mg/g, 170 mg/g and 75mg/g of the crude drug powder and the concentration of *Centella asiatica*, Linn. is 83mg/g, 183 mg/g and 68mg/g of the crude drug powder and the concentration of *Murraya koenigii*, Linn. is 95 mg/g, 162 mg/g and 70 mg/g respectively. (Graph - 1).

Graph 1 – Total Carbohydrate, Protein and Phenol Content



3.3 ANTICANCEROUS ACTIVITY

Anticancerous activity is observed in *Andrographis paniculata*, Nees, *Centella asiatica*, Linn *and Murraya koenigii*, Linn. Anticancerous activity is more observed in Murraya koenigii, L. as compared to *Andrographis paniculata*, Nees. and *Centella asiatica*, Linn. (Table – 4, Plate 5 and Graph - 2).

Table 4 - Anticancerous Activity of the plants

Crude drug concentration (µg\ml)	% of cell death in the extracts of *Andrographis paniculata*,Nees	% of cell death in the extracts of *Centella asiatica*,L	% of cell death in the extracts of *Murraya koenigii*,L
200	55	63	78
100	42	51	53
50	28	35	41
20	9	11	15
10	3	5	8

Graph 2 – Anticancerous Activity

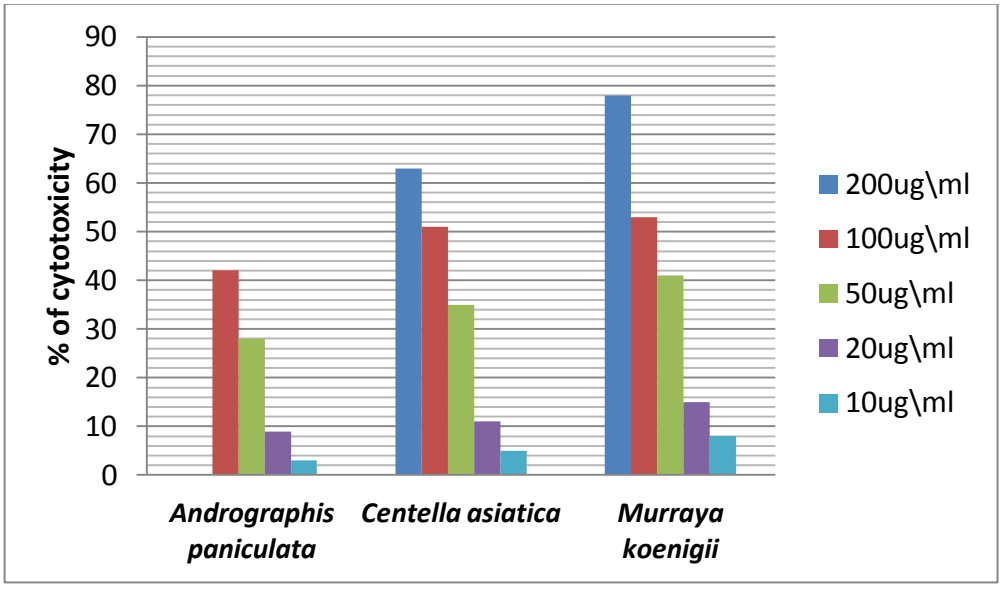
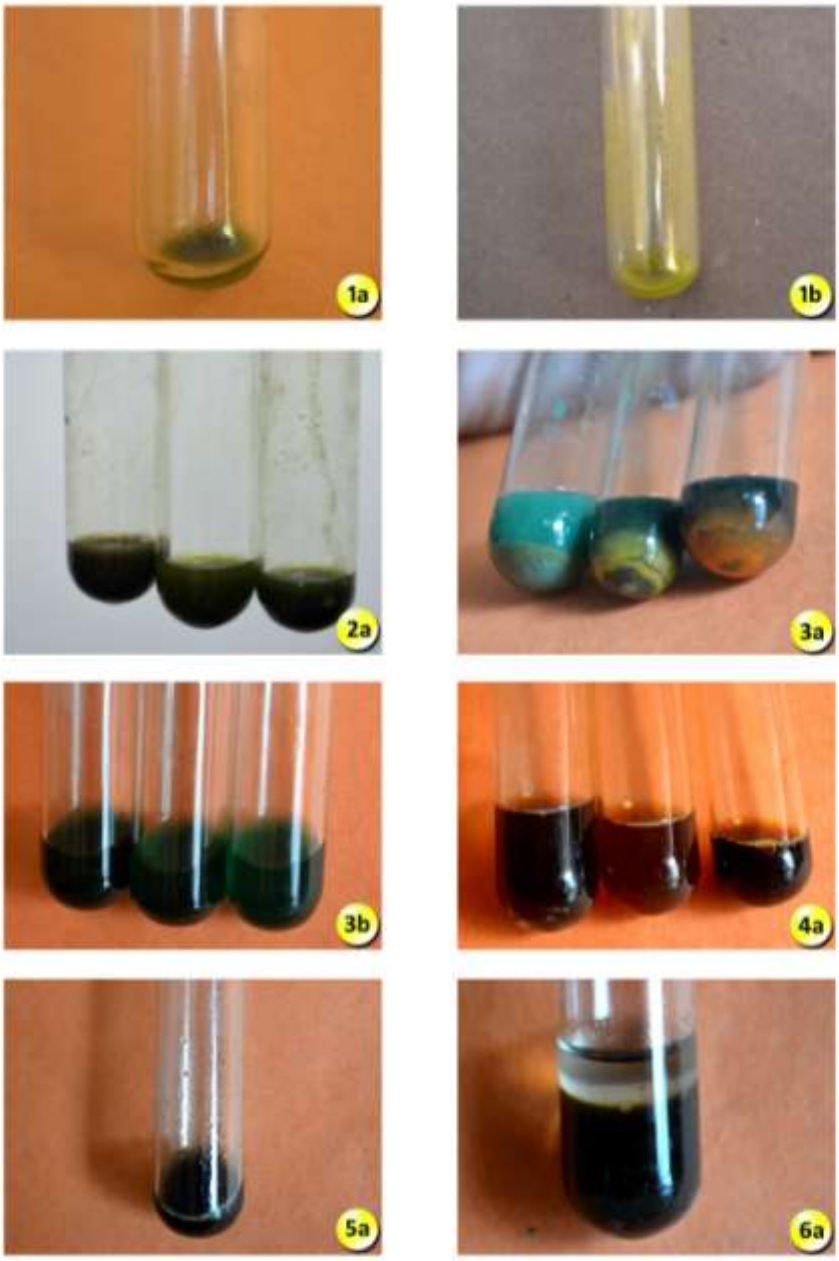


PLATE 1

Plant Materials Used

(1, 2) *Andrographis paniculata*, Nees. (3, 4) *Centella asiatica*, L. (5, 6) *Murraya koenigii*, L.

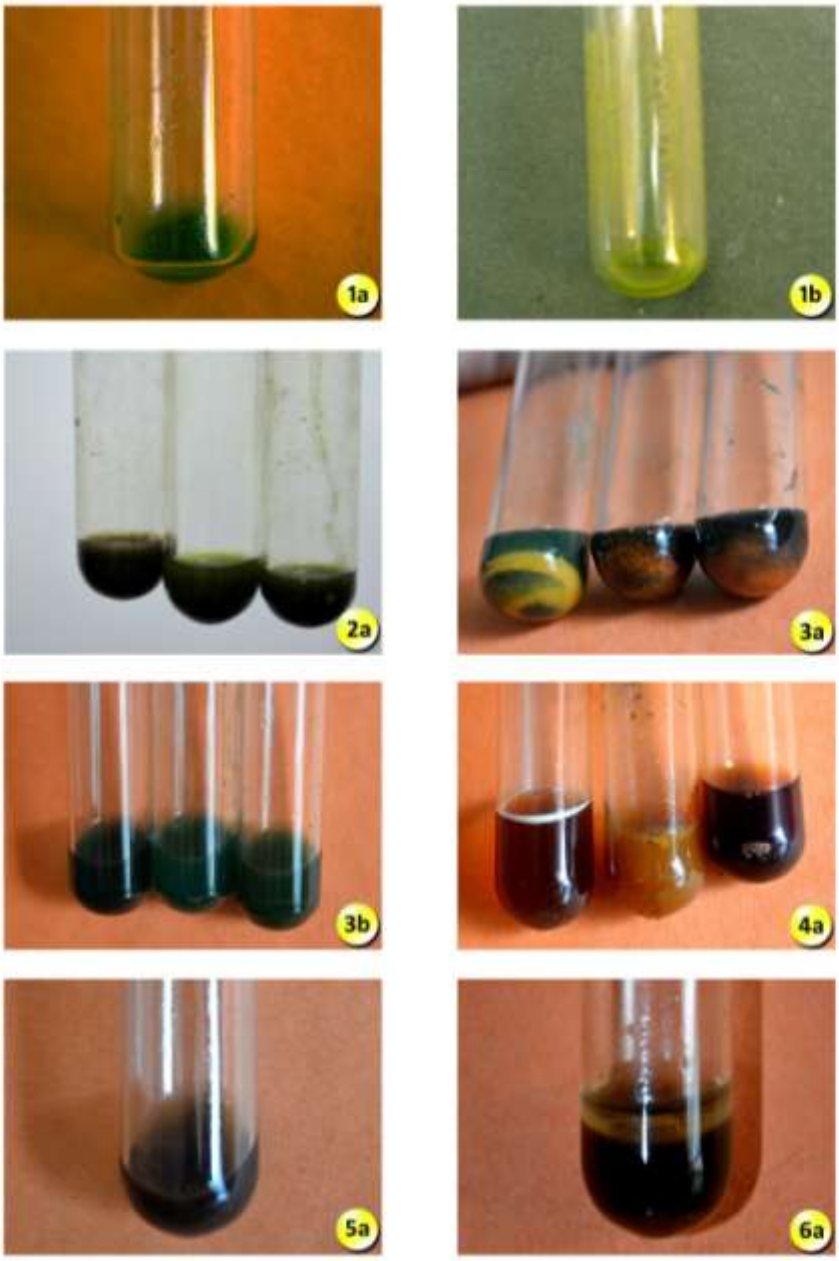
PLATE 2



Phytochemical analysis of *Andrographis paniculata*, Nees.

1 - Alkaloid (a) Wagers test (b) Mayers test; 2 - Tannin & Phenol (a) Ferric chloride test;
3 - Carbohydrate (a) Fehling test (b) Benedicts test; 4 - Protein (a) Xanthoprotein test;
5 - Flavanoid (a) Pew test; 6 - Glycosides (a)Borntragers test.

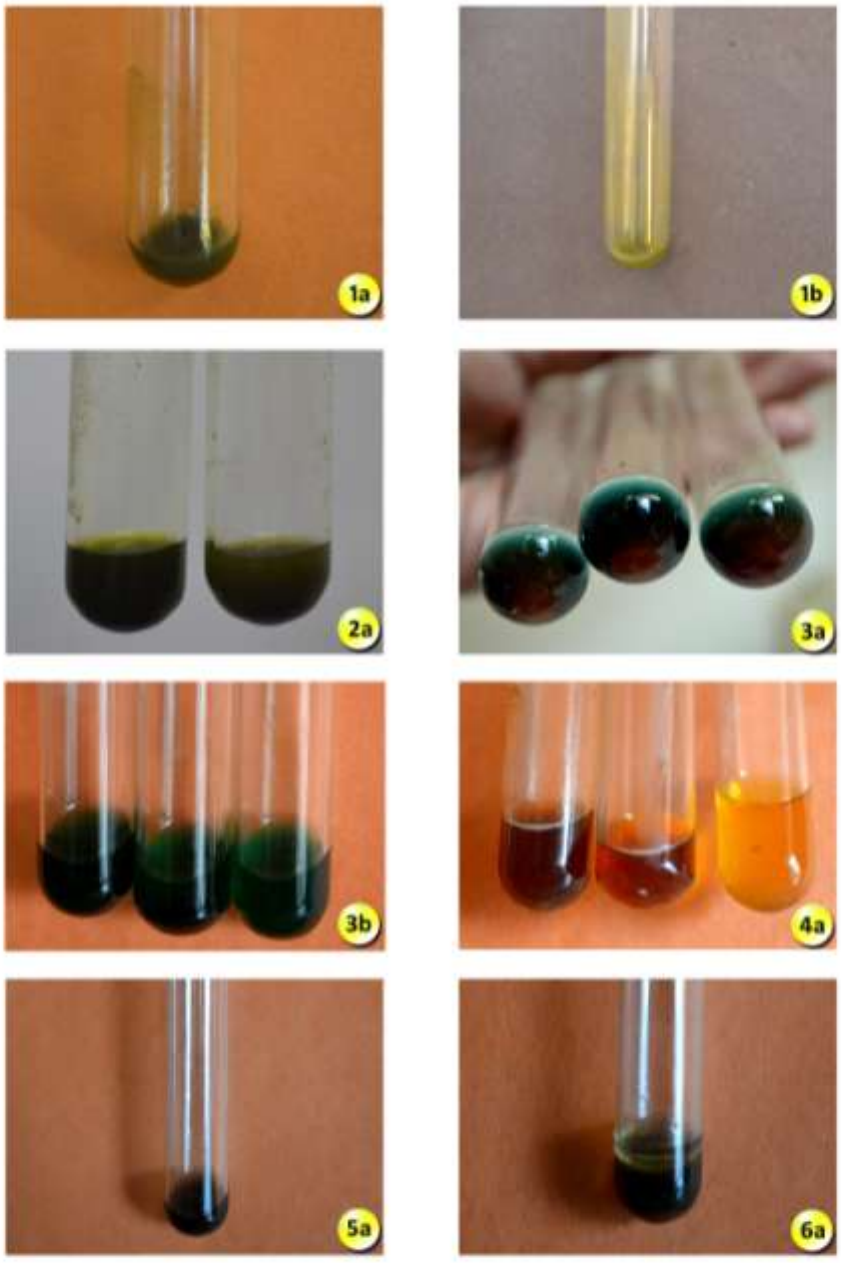
PLATE 3



Phytochemical analysis of *Centella asiatica, L.*

1 - Alkaloid (a) Wagers test (b) Mayers test; 2 - Tannin & Phenol (a) Ferric chloride test;
3 - Carbohydrate (a) Fehling test (b) Benedicts test; 4 - Protein (a) Xanthoprotein test;
5 - Flavanoid (a) Pew test; 6 - Glycosides (a)Borntragers test.

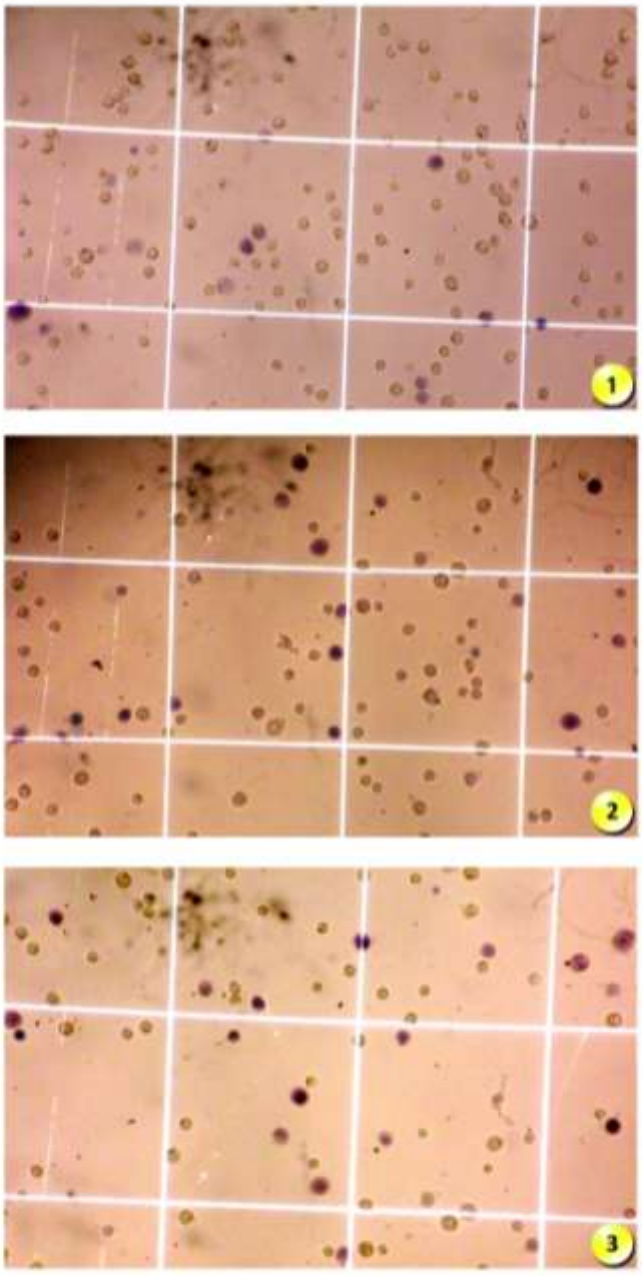
PLATE 4



Phytochemical analysis of *Murraya koenigii*, L.

1 - Alkaloid (a) Wagers test (b) Mayers test; 2 - Tannin & Phenol (a) Ferric chloride test;
3 - Carbohydrate (a) Fehling test (b)Benedicts test; 4 - Protein (a) Xanthoprotein test;
5 - Flavanoid (a) Pew test; 6 - Glycosides (a)Borntragers test.

PLATE 5



Anticancerous Activity

(1) *Andrographis paniculata*, Nees. (2) *Centella asiatica*, L. (3) *Murraya koenigii*, L.

IV. Discussion

The phytochemical analysis and anticancerous activity of *Andrographis paniculata,* Nees., indicate that the presence of active compounds such as carbohydrates, proteins, tannin and phenols in different extraction such as aqueous, methanol and ethanol. In this analysis,saponin is absent. Alkaloid is a major content in this plant, which gives the bitter principle to this plant. So it is also called as "King of Bitters". According to the study of Kalaivani *et al*., (2012) evaluation of ethanolic leaf extract of *Andrographis paniculata*, Nees. revealed that tetradecanoic, phytol, squalene, sitosterol compounds were having anticancer properties . The present study shows the anticancerous activity in methanolic extract of whole plant. The phytochemical analysis of *Centella asiatica*,L., indicate that the presence of active compounds such as carbohydrates, proteins, tannin, phenols, flavanoids, glycosides and coumarins in different extraction such as aqueous ,methanol and ethanol. Researchers at the Amala Cancer Research Centre in Kerala, India,tested both a crude extract of *Centella asiatica*,Linn. (CE) and its partially purified fractions (AF) for their antitumor activity. AF significantly inhibited the proliferation of the transformed cell lines in Ehrlich ascites tumor cells and Dalton's lymphoma ascites tumor cells with no toxic effects on normal human lymphocytes. (Babu et al, 1995). As per the present study, crude extract of *Centella asiatica*, L. shows the anticancerous activity . The phytochemical analysis of *Murraya koenigii,L.* , indicate that the presence of active compounds such as carbohydrates,proteins,tannin,phenols,flavanoids,coumarins and glycosides in different extraction such as aqueous ,methanol and ethanol.In this analysis,saponin is absent.The study of Anu *et al* .(2013) reveals presence of protein ,resin, steroids, tannins, glycosides, reducing sugar ,carbohydrates, phenol in aqueous and methanol extract of *Murraya koenigii,L.* As per the present study,saponin is absent in aqueous and methanol extract. Phenol and tannin is absent in aqueous extract,but it is present in methanol and ethanol extract.

V. Conclusion

Medicinal plants are the backbone of the traditional system of medicine. Indian medicinal plants are considered a vast source of several pharmacologically active principles and compounds. In the present investigation an attempt has been made for the pharmacological evaluation of *Andrographis paniculata,* Nees., *Centella asiatica,* Linn. *and Murraya koenigii,* Linn.. The pharmacological evaluation consists of phytochemical analysis and anticancerous activity of three plants were done. This research work states that the presence of alkaloids,flavanoids,sugars,coumarins etc in the methanol, ethanol and water extract of *Andrographis paniculata,* Nees. , *Centella asiatica,* Linn. *and Murraya koenigii,* Linn. In this study ,the effect of methanol extract of *Andrographis paniculata,* Nees. , *Centella asiatica,* Linn. *and Murraya koenigii,* Linn. shows the anticancerous activity. Anticancerous activity is more observed in Murraya koenigii, L. as compared to *Andrographis paniculata,* Nees . and *Centella asiatica,* Linn.*(Murraya>centella>andrographis).* In conclusion, the results of this study indicated that methanol is the better solvent for the extraction of phytocompounds.

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